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Macroorchidism due to Autonomous Hyperfunction of Sertoli Cells and $G_s\alpha$ Gene Mutation: An Unusual Expression of McCune-Albright Syndrome in a Prepubertal Boy

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ABSTRACT

We report an unusual observation of a 3.8-yr-old boy with McCune-Albright syndrome (MAS) associated with abnormal prepubertal testis enlargement and no sexual precocity. Physical examination showed café-au-lait skin lesions, enlarged testes, prepubertal sized penis, and no pubic or axillary hair. Skeletal radiography disclosed fibrous dysplasia. The serum testosterone level was 0.58 nmol/L and remained below 1.4 nmol/L during the 4-yr follow-up. By contrast, serum inhibin B and anti-Mullerian hormone concentrations were abnormally increased up to 255 pg/mL (childhood range, 35–180) and 792 pmol/L (childhood range, 309–566), respectively. The LH response to a GnRH test was in the prepubertal range, whereas the FSH response was blunted. This abnormal hormone concentration profile indicates autonomous hyperfunction of Sertoli cells, with no evidence of Leydig cell activation. Testicular histology showed tubules with marked Sertoli cell hyperplasia and very rare germinal cells, and interstitial tissue containing mesenchymal cells but no mature Leydig cells. DNA sequence analysis from bone and testis tissues detected the known activating mutation in MAS that results in replacement of Arg by His at codon 201 of the $G_s\alpha$ protein. Other endocrine tests showed excessive GH secretion and moderate adrenal androgen hypersecretion.

These findings are consistent with the occurrence of an activating mutation of the $G_s\alpha$ gene mainly expressed in Sertoli cells and weakly expressed or absent in Leydig cells. Abnormal prepubertal testicular enlargement extends the clinical spectrum of MAS, suggesting that determination of serum inhibin B and anti-Mullerian hormone should be considered in boys with this syndrome. This observation demonstrates the usefulness of detailed molecular and biological investigations in atypical cases of MAS. (*J Clin Endocrinol Metab* **86:** 1778–1781, 2001)

THE MCCUNE-ALBRIGHT syndrome (MAS), which occurs far more frequently in girls than in boys, is classically characterized by the triad of polyostotic fibrous dysplasia, café-au-lait skin lesions, and GnRH-independent sexual precocity (1–4). Other various endocrinopathies have been described in this syndrome (3–5). Somatic gain of function mutations in the gene coding for the $G_s\alpha$ protein have been found in affected tissues of patients with MAS, including the ovary, testis, adrenal gland, pituitary gland, thyroid gland, skin, and bone (3–7). $G_s\alpha$ protein is implicated in the signaling pathway of numerous membrane-bound receptors, including receptors for several hormones. Substitution of Arg^{201} by His or Cys decreases the guanosine triphosphatase activity of $G_s\alpha$, leading to constitutive activation of G_s protein. Somatic mosaicism of $G_s\alpha$ protein results in an admixture of affected and normal cells and accounts for the variation in the site and degree of involvement of different tissues among patients with MAS (6).

Sexual precocity in MAS is due to activation of gonadotropin receptor signaling in the absence of interactions between gonadotropins and their receptors (6). Whereas precocious puberty is the common initial manifestation in girls, it has been reported in only 15% of affected boys (5). Subjects have enlarged testis in addition to signs of sexual precocity, and serum testosterone levels are in the pubertal range, contrasting with low secretion of LH and FSH (8–14).

We report an unusual clinical expression of MAS in a 3.8-yr-old boy presenting with abnormal prepubertal testicular enlargement but no sexual precocity. The hormonal, histological, and molecular investigations revealed testicular

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TABLE 1. Hormonal evaluation of the patient

	Age (yr)					
	3.8	5	6	6.6	7.5	Normal values
Testosterone (nmol/L)	0.58	0.95	0.84	0.84	1.40	<1.1
S-DHEA (nmol/L)	1700	1200	1200	2000	2000	<800
LH (IU/L)						
Basal	<0.2			<0.2	<0.2	0.10–1.30
Peak (GnRH test)	1.6			0.9		0.90–4.80
FSH (IU/L)						
Basal	0.3			0.1	<0.06	0.20–1.60
Peak (GnRH test)	0.7			0.5		1.50–6.10
Inhibin B (pg/mL)	242		255	246		35–180
AMH (pmol/L)	448	726	792	544	286	309–566
IGF-I (ng/mL)	435	631	624	736	498	25–250
GH (μg/L)						
Basal	10.4			8.2		
Nadir (OGTT)	9.9			5.6		<2
PRL (ng/mL)	20	16		19		<20

OGTT, Oral glucose tolerance test. Normal values are those corresponding to the age of the patient.

autonomous hyperfunction restricted to Sertoli cells with no activation of Leydig cells.

Materials and Methods

The subject and his family gave informed consent for this study.

Laboratory assays

Inhibin B was measured by means of a solid phase sandwich assay (Serotec, Oxford, UK) as previously described (15). Inhibin A exhibited 1% cross reactivity in the inhibin B assay. Intraassay precision was 7.4% and 4.2% at levels of 44 and 225 pg/mL, respectively. The sensitivity was 6 pg/mL. Reference values in boys aged 3–8 yr are 35–180 pg/mL. Anti-Mullerian hormone (AMH) was measured with a solid phase sandwich assay using reagents provided by Immunotech/Beckman Coulter, Inc. (Villepinte, France) (16, 17). There was no cross-reactivity of related proteins, including transforming growth factor-β. Intraassay precision at 245 and 1106 pmol/L was 5.1% and 4.9%, respectively. The sensitivity was 0.7 pmol/L. Reference values in boys aged 4–7 yr were 309–566 pmol/L (17). RIAs were used to measure plasma concentrations of testosterone, dehydroepiandrosterone sulfate, cortisol, and aldosterone (Immunotech/Beckman Coulter, Inc.; Diagnostics Systems Laboratories, Inc., Webster TX). LH and FSH were measured by immunoradiometric assays (IRMAs; Immunotech/Beckman Coulter, Inc.; Coat-a-Count, Diagnostic Products, Los Angeles, CA) at baseline and after administration of 100 μg GnRH (gonadorelin, Ferring Pharmaceuticals Ltd., Gentilly, France). GH was measured by IRMA (CIS-Bio International, Gif-sur-Yvette, France). Total insulin-like growth factor I measurements were performed by IRMA after acid-ethanol extraction, PRL and hCG were measured by IRMA (Immunotech/Beckman Coulter, Inc.).

Identification of $G_s\alpha$ gene mutation

A bone biopsy was performed at age 6.2 yr during surgery for a pathological femoral fracture. A testicular biopsy was obtained at age 7.5 yr by a surgical procedure; a three-cornered section, including albuginea, was made opposite the epididymis. The biopsy was divided into two fragments: a fragment consisting only of testicular parenchyma was snap-frozen for further molecular analysis, and the remaining tissue was used for histological studies. Enzymatic amplification was performed on DNA extracted from bone and testis tissues. A method previously described for selective enrichment of mosaic Arg^{201} mutations was used, with minor modifications (18). Briefly, primer containing mismatch was used to generate a PCR product from the normal allele (normal codon 201) that is susceptible to *Eag*I digestion, whereas that from the mutant allele (mutated codon 201) is resistant to digestion. Successive steps of PCR, enzymatic digestion, and nested PCR allowed selective enrichment of the product from the mutant allele. Experiments were limited to two steps of *Eag*I treatment and nested PCR to reduce the risk of contamination. In addition, negative controls (no DNA) and normal DNA were included in all series of experiments. PCR products were purified and sequenced with the antisense primer using the ABI Prism Dye terminator sequencing kit (Perkin-Elmer Corp., Foster City, CA). Sequencing reactions were performed twice with two different PCR products.

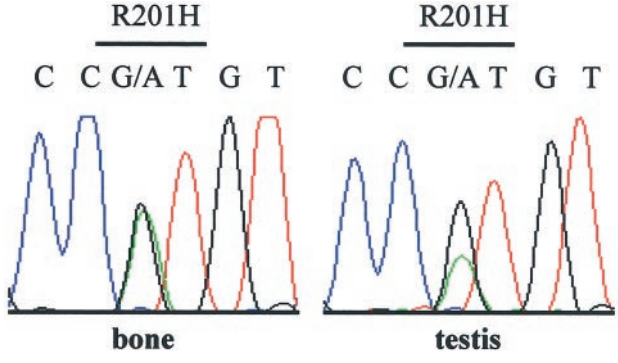

FIG. 1. Partial chromatograms of exon 8 of the $G_s\alpha$ gene showing the somatic R201H mutation in DNAs extracted from bone (*left panel*) and testis (*right panel*).

Histological and immunocytochemical studies of testicular tissue

Testicular tissue was fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer, dehydrated in a graded series of ethanols, and embedded in paraffin; 5-μm sections were cut and processed for routine hematoxylin-eosin staining. Immunocytochemistry was performed using the antigen retrieval technique by microwaves, as previously described (19), for AMH and inhibin β_B-subunit. Antigen retrieval was not necessary for the detection of 3β-hydroxysteroid dehydrogenase.

Results

Case report

A boy aged 3 yr, 10 months was admitted to our clinic because of right hip pain and alteration of gait. Physical examination revealed several café-au-lait spots on the back. The volume of the right testis was 9 mL, and that of the left was 7 mL. Contrasting with the pubertal volume of testes, the penis was infantile in size (4.5 $\times$ 1.5 cm), and there was no

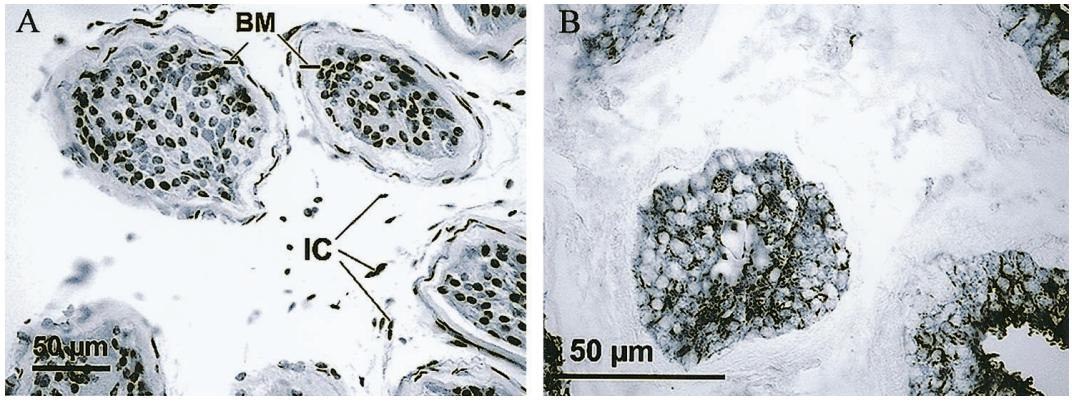

FIG. 2. Histological and immunocytochemical study of testicular tissue. *Bar*, 50 μm. A, Sample representing the features of testicular tissue observed in 42 of 50 slides analyzed in this study. Seminiferous tubules show no lumen and are filled with Sertoli cells, the basement membrane (BM) is enlarged, and only interstitial cells (IC) with mesenchymal or fibroblastic appearance are seen in the interstitial tissue (hematoxylin-eosin stain). B, Immunohistochemistry for inhibin β_B-subunit shows an intense reaction in the cytoplasm of Sertoli cells.

pubic or axillary hair. The height was 108 cm (+1.7 SD score), and growth velocity was 9 cm/yr (+2.7 SD score). There was no familial history of precocious puberty. Skeletal radiography showed numerous cystic areas of rarefaction in iliac and femoral bones, and thickening and expansion of the base of the skull. Bone age was 5 yr. A presumptive diagnosis of MAS was made.

Initial hormonal evaluation showed a prepubertal serum testosterone level of 0.58 nmol/L (Table 1). After the iv administration of GnRH, the peak serum LH level was in the prepubertal range, whereas the peak FSH level was blunted. By contrast, serum inhibin B was increased to 242 pg/mL, a pubertal level indicating Sertoli cell activation. Serum hCG was not detectable.

During the 4-yr follow-up, no pubic or axillary hair appeared. The penis remained infantile, whereas testes were pubertal in size. Serum testosterone concentrations remained below 1.40 nmol/L, contrasting with pubertal levels of inhibin B (167–255 pg/mL) and high levels of AMH (546–792 pmol/L; Table 1).

Growth rate was accelerated, and serum insulin-like growth factor I was abnormally elevated (Table 1). Excessive GH secretion was diagnosed, as high serum GH levels failed to decrease after an oral glucose tolerance test. The child received somatostatin analogs from the age of 6.6 yr.

Moderate adrenal hyperfunction was diagnosed as the serum dehydroepiandrosterone sulfate concentration was increased, with normal levels of cortisol and aldosterone (Table 1). The PRL concentration and serum TSH and free T_4 levels were in the normal range. Pituitary magnetic resonance imaging and adrenal computed tomography scan were normal.

Molecular study

Bone and testicular biopsies were performed, and DNA was extracted from the tissue specimens. Sequencing showed the presence of a guanine to adenine transversion leading to an Arg-His substitution at position 201 (Fig. 1) in bone and testis tissues. Both normal and mutant sequences of the $G_s\alpha$ gene were present, indicating a somatic mutation and thus a mosaicism of normal and abnormal cells as observed in MAS. Due to the enrichment method used, evaluation and comparison of the amounts of mutant and wild-type DNAs were not applicable.

Histological study

Testicular histology showed a majority of seminiferous tubules with slightly increased diameter, but no lumen, filled with hyperplastic Sertoli cells; germ cell number was significantly reduced. The basement membrane was enlarged, and the number of peritubular cell layers was increased. The interstitial tissue contained mesenchymal cells, but showed no Leydig cell maturation (Fig. 2A). The immunocytochemical study showed a homogeneously positive reaction in Sertoli cells for inhibin β_B-subunit (Fig. 2B) and AMH (not shown). To probe an eventual steroidogenic activation of interstitial cells, we performed immunocytochemistry for enzyme 3β-hydroxysteroid dehydrogenase; no positive reaction was observed. A positive reaction was observed in the Leydig cells from a section of pubertal testis taken from our tissue library and used as a control (not shown).

Discussion

The clinical presentation of MAS in the present report is unusual, as prepubertal testicular enlargement with no sexual precocity revealed testicular autonomous hyperfunction restricted to Sertoli cells, with no evidence of activation of Leydig cells. Pubertal serum levels of inhibin B indicated Sertoli cell hyperfunction, which exerted a negative feedback on FSH secretion. Increased serum AMH levels were also a marker of increased Sertoli cell number or function (19). By contrast, Leydig cell hyperfunction was unlikely, because the testosterone concentration remained below 1.4 nmol/L. Testicular histology and immunocytochemistry showed Sertoli cell hyperplasia, with no maturation or steroidogenic activation of Leydig cells. Molecular study on testicular biopsy identified the activating $Arg^{201}His$ mutation of the $G_s\alpha$ gene.

Only a dozen cases of sexual precocity in boys with MAS have been reported to date (8–14). Subjects had enlarged testes and penis in addition to premature pubic hair. Serum testosterone levels were in the pubertal range, whereas the responses of gonadotropins to GnRH were weak (8). Testic-

ular histology showed seminiferous tubules lined by Sertoli cells and germ cells, whereas mesenchymal cells underwent maturation to Leydig cells (8, 14). The full process of spermatogenesis has been described in patients as young as 6 yr (8). These hormonal data and testicular findings appeared similar to those in boys with familial testotoxicosis and indicated LH receptor signaling pathway activation (14). Molecular studies performed on testis tissue in some cases revealed the expected mutation of the $G_s\alpha$ gene in the specimens (6, 7, 13). One report, although labeled as precocious pubertal development, showed many similarities with the present case (12). The 6.5-yr-old affected boy had enlarged testes, contrasting with infantile penis and no pubic hair. Serum testosterone level and LH and FSH responses to GnRH were in the prepubertal range. Testicular histology showed mature tubules exhibiting spermatogenesis and interstitial tissue containing rare Leydig cells. Although inhibin B and AMH were not measured, prevailing Sertoli cell activation was likely.

The boy had tall stature, accelerated growth rate and advanced bone age. In the absence of circulating testosterone in the pubertal range, these features were probably explained by the excessive GH secretion and the abnormally increased adrenal androgen concentration. Pituitary and adrenal involvement has been previously reported in MAS (3–7, 11, 12).

Several explanations may account for the restriction of testicular autonomous hyperfunction to Sertoli cells in the present observation. One possibility is that somatic mutation of the $G_s\alpha$ gene was present in Sertoli cells but not in Leydig cells (6). Although the mutation is believed to occur early in development of the embryo, the origin of Sertoli and Leydig cells is controversial, and recent reports in mouse indicated that the two populations show somewhat different ontogenies (20, 21). Alternatively, imprinting of the $G_s\alpha$ gene, which has been hypothesized from studies on pseudohypoparathyroidism type I (22–24), may lead to cell-specific expression of the mutated gene; the allele encoding the activated $G_s\alpha$ protein in our patient may be expressed in Sertoli cells, but not in Leydig cells. Another explanation arises from mouse models showing that overexpression of AMH exerts an inhibitory effect on Leydig cell maturation and function (25, 26). In our patient, strong expression of the activating mutation in Sertoli cells produced high levels of AMH, which may have counteracted the effect of weak expression of the mutation in Leydig cells.

Testicular histology was abnormal in this patient. The seminiferous tubules were filled with hyperplastic Sertoli cells, but germ cell number was reduced. The interstitial tissue contained mesenchymal cells, but there was no Leydig cell maturation. This is in sharp contrast with testicular findings in boys with MAS and sexual precocity. Whereas potent Leydig cell activation results in germinal cell maturation, isolated Sertoli cell activation may be detrimental for germinal cell maintenance.

In conclusion, we described a new endocrinopathy in MAS: abnormal testicular enlargement with no sexual precocity resulted from autonomous testicular hyperfunction restricted to Sertoli cell with no activation of Leydig cell. Determination of serum inhibin B and AMH in addition to testosterone and gonadotropins should be considered in boys with MAS to characterize potential testicular involvement. This observation demonstrates the usefulness of detailed molecular and biological investigations in atypical cases of MAS.

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